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Fructose exposure during gestation and lactation altered hepatic selenoprotein expression, oxidative balance and metabolic profile in female rat pups

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ABSTRACT

Fructose-rich diets, an experimental model of induced metabolic syndrome (MS), affects pregnant and lactating rat dams, and moreover, it leads to an imbalance in their offspring's metabolic profile. Selenium (Se) homeostasis and body distribution is also altered. Selenoproteins: Glutathione peroxidase (GPx) and selenoprotein P (SelP) appear to play a role in MS. To evaluate selenoproteins' implication in the transmission of this pathology to the progeny, hepatic Se deposits, GPx activity, biomolecular oxidation and selenoproteins and AMPK expression were measured in the offspring of dams exposed to a fructose-rich diet (65%). Se intake and liver selenoproteins are affected by fructose exposition during early nutrition. Female fructose pups show a repletion of Se and oxidation in liver, higher GPx activity and expression of hepatic GPx1 and SelP, related to a lower activation of AMPK and serum insulin levels. Fructose exposure during early nutrition negatively alters selenoproteins, oxidation and metabolism in female pups.

1. Introduction

Metabolic syndrome (MS) is defined as a cluster of risk factors including central obesity, insulin resistance (IR), raised blood pressure and dyslipidemia that predispose sufferers to cardiovascular diseases and diabetes (Day, 2007). This syndrome also appears among pregnant women, affecting both mother and offspring (Harreiter, Dovjak, & Kautzky-Willer, 2014; Zou et al., 2012). In this context, it is also known that fructose-rich diets, an experimental model of induced MS, affects pregnant and lactating dams in a more pronounced way that in unmated rats (Zou et al., 2012). This leads to an important imbalance in their offspring's metabolic profile with sex differences due to the changes in fructose exposition during early nutrition programming (gestation and lactation periods) (Nogales et al., 2017; Ojeda et al., 2016). Those studies also found that Selenium (Se) homeostasis and body distribution were altered in fructose-exposed rat dams and offspring.

Se is a trace element that plays its biological functions by forming part of 25 selenoproteins (Carreras, Ojeda, & Nogales, 2015), among which the family of the antioxidant enzymes glutathione peroxidase (GPx), and the Se plasma transporter selenoprotein P (SelP) have been recently related to IR and MS. However, both infra- and supra-protein regulations have been implicated in the development of this pathology (Seale et al., 2012; Wang et al., 2014).

As the excess of reactive oxygen species (ROS) generated causes damage to mitochondrial components, oxidative stress is involved in the pathogenesis and etiology of MS. Moreover, it is known that in MS patients oxidative stress is amplified by a concomitant antioxidant deficiency, where superoxide dismutase (SOD) and GPx play a pivotal role (Bonomini, Rodella, & Rezzani, 2015; Camargo et al., 2014). GPx1 and SelP upregulation usually has beneficial antioxidant effects, but GPx1 also diminishes intracellular ROS, which are needed in insulin signaling. When insulin reaches its receptor, ROS is liberated. These species deactivate the insulin-signaling inhibitors' phosphatase protein (PTEN) and protein tyrosine phosphatase 1B (PTP-1B) which contribute to the insulin signaling process (Brigelius-Flohé and Maiorino, 2013; Wang et al., 2014; Zhou, Huang, & Lei, 2013), a process prevented by the selenoproteins' antioxidant activity. In agreement with the above, Steinbrenner (2013) showed that GPx1 and/or SelP inhibited phosphorylation (activation) of key mediators in energy metabolism such as protein kinase B (Akt) and adenosine monophosphate-activated protein kinase (AMPK) in liver and/or skeletal muscle.

Contributing to this complexity of selenoprotein implications in MS, human *gpx4* gene variants have been associated with obesity and cardiovascular disease in epidemiological studies (Crosley et al., 2013; Ruperez et al., 2014). In this context, Katunga et al. (2015) have found

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that GPx4 deficiency in obesity leads to enhanced lipid peroxidation and carbonyl stress in liver, exacerbating IR and steatosis.

For this reason, the aim of this study is to evaluate hepatic oxidative profile, and GPx1, GPx4 and SelP expression together with the ratio p-AMPK/AMPK in the offspring of MS rat dams in order to further knowledge of selenoprotein's role in MS and early nutrition programming after high fructose consumption. As metabolic profile has been recently demonstrated to be affected by sex hormones during gestation and/or lactation, sex differences were also analysed.

2. Materials and methods

2.1. Animals

Male and female Wistar rats (Centre of Production and Animal experimentation, Vice-rector's Office for Scientific Research, University of Seville) weighing approximately 150–200 g, were randomised into two groups: control (C) and fructose (F) groups. This later group presented MS which was induced with a rich fructose diet (65%). All rats received drinking water and diet (control or rich fructose diet) *ad libitum* during three week before mate (to induce MS in F group), and then, during gestation (3 weeks) and lactation (3 weeks) periods. In four week, male (n = 3) and female (n = 6) rats were mated to obtain the first-generation offspring for each group. Pregnant female rats were inspected daily by the presence of the vaginal plug, which indicated day zero of pregnancy; at this moment pregnant rats were housed individually in plastic cages. The day of parturition, which occurs spontaneously three weeks after coitus, was designated as day 1 of lactation. The offspring number was reduced to 8 per mother at parturition (four males and four females, when this was possible). The experiments were performed on the offspring of all two groups to 21d postpartum. In this study, we have used 8 pups per group to measure all the parameters cited below. These 8 pups represent all the litters, as a maximum of 2 rats per litter, and were allocated to each group taking into account the sex. The animals were kept at an automatically controlled temperature (22–23 °C) and a 12-h light-dark cycle (9:00–21:00). Animal care complied with the Guide for the Care and Use of Laboratory Animals (National Academy Press, Washington, D.C., 1996). Animal studies have been approved by the Seville University ethics committee.

2.2. Diets and nutritional controls

The diets of these rats were prepared according to The Council of the Institute of Laboratory Animal Resources (ILAR, 1979) which details known nutrient requirements for most of the common laboratory animals. The diet of control group contained sucrose and starch, while high fructose diet contained only fructose (65%) as carbohydrate source. Both diets contained 0.1 ppm of Se as anhydrous sodium selenite (an inorganic compound) (Panreac, Barcelona, Spain).

Body weights of the rats were determined once a week while that the amount of food and liquid consumed by rats were monitored daily until the end of the experimental period. At end of experiment, before of sacrifice, cranium-caudal length was measured using a metric calliper. Body weight and length were used to determine the body mass index (BMI), which was calculated according to the formula: Body weight (g)/length^2 (cm^2). All measures were taken at 9:00 am to avoid changes due to circadian rhythms.

2.3. Metabolic profile

Glucose, triglycerides and cholesterol were determined in dams and their pups using test strips Accutrend (Roche, Spain) from tail blood. Serum insulin was determined by using a rat insulin ELISA kit (BioVendor GmbH, Heidelberg, Germany) according to the manufacturer's instructions. Fasting glucose and insulin serum concentrations were used to calculate the model homeostasis assessment of insulin resistance index (HOMA-IR) according to the following formula: (Fasting glucose concentration X Fasting insulin serum concentration)/405).

2.4. Samples

The amount of milk consumed by the offspring at the end of the lactation period (days 19 and 20) was estimated by subtracting the weight of the pups obtained immediately prior to returning them to the dam from their weight after 30 min of suckling. In order to obtain the maximum amount of milk at day 21 of lactation to measured Se content, 3 h after removing the litters from their mothers, the dams were anesthetized with urethane (28% w/v, 0.5 ml/100 g of body weight) and milk samples were immediately collected. The milk was obtained by gently massaging the area around each of the 12 mammary glands and then pressing upward from the base of the gland towards the nipple. The amount of milk collected was around 1–1.5 ml per dam. Therefore, at the end of the experimental period, dams, and latter their pups were anesthetized with intraperitoneal urethane. Blood samples were obtained by heart puncture and collected in tubes. The serum was prepared using low-speed centrifugation for 15 min. at 1300 × *g*. The abdomen was opened by a midline incision and liver was removed, debrided of adipose and connective tissue in ice-cold saline, weighed and stored at −80 °C prior to biochemical determinations.

2.5. Selenium analysis

Selenium levels were determined by graphite-furnace atomic absorption spectrometry, using a PerkinElmer AAnalyst™ 800 high-performance atomic absorption spectrometer with WinLab32 for AA software, equipped with a Transversely Heated Graphite Furnace (THGA) with longitudinal Zeeman-effect background corrector and an AS-furnace autosampler (PerkinElmer, Überlingen, Germany). The source of radiation was a Se electrodeless discharge lamp (EDL). The instrumental operating conditions and the reagents are the same that we have used in the previous paper Ojeda et al. (2009). Samples: serum samples were diluted fivefold in 0.2% v/v HNO_3 and 0.2% Triton X-100 solutions, liver and milk samples were weighed and digested in a sand bath heater (OVAN, Badalona, Spain) with nitric acid for 72 h, and perchloric acid and chlorhydric acid (6 N) were added.

2.6. Antioxidant enzymes and oxidative stress markers

In order to measure the activity of antioxidant enzymes as well as the oxidation of lipids and protein, liver tissue samples were homogenized (Pobel 245,432, Spain) in a sucrose buffer (15 mM Tris/HCl, pH 7.4, 250 mM sucrose, 1 mM EDTA and 1 mM DTT) in an ice bath. The homogenate was centrifuged at 3000 rpm for 10 min at 4 °C. The resulting supernatant was employed for the biochemical assay. Glutathione peroxidase (GPx) activity was determined by the method of Lawrence and Burk (1996), in which GPx catalyzes the oxidation of glutathione by hydrogen peroxide. The glutathione reductase (GR) was determined by using the methods of Worthington and Rosemeyer (1976). Lipid peroxidation was evaluated by the method based on the reaction between malondialdehyde (MDA) and thiobarbituric acid (Draper and Hadley, 1990). Hepatic proteins' oxidation was measured according to a method based on the spectrophotometric detection of the reaction of 2,4-dinitrophenylhydrazine with protein carbonyl (PC) to form protein hydrazones (Reznick and Packer, 1994). The protein content of the samples was determined by the method of Lowry, Rosebrough, Farr, and Randall (1951), using bovine serum albumin as the standard.

2.7. Immunoblotting assays

To determine the expression of the hepatic selenoproteins GPx1,